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Mechanistic Toxicology Research and Biologically-Based Modeling: Partners for Improving Quantitative Risk Assessments

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In quantitative risk assessments, the "dose-response assessment" provides information necessary for predicting the expected incidence of response at various exposure levels in laboratory animals and people. Because of the importance in distinguishing between exposure levels and dose to critical target tissues, the CIIT research program emphasizes the use of a more comprehensive terminology, "exposure-dose-response assessment." The present uncertainties surrounding conventional low-exposure level and interspecies extrapolation of response incidence can be improved when the results of mechanistic toxicological research are interpreted within the context of biologically-based models. This article examines the progress in developing biologically-based simulation models of toxicant disposition, toxicant-target tissue interaction and tissue responses, and the role these models play in providing a risk assessment orientation for CIIT research efforts.

The assessment of potential health risks associated with human exposure to chemicals is performed in four steps: (1) hazard identification, (2) exposure assessment, (3) dose-response assessment, and (4) risk characterization (National Academy of Sciences, 1983). Once the toxic effects of a chemical are identified ("hazard identification"), the health risks associated with human exposure to that chemical are characterized ("risk characterization") by combining quantitative information on its exposure levels ("exposure assessment") and on the dose-response relationships for various toxic effects ("dose-response assessment").

The original NAS report used the expression "dose-response assessment" to refer to the process of estimating the expected incidence of response for various exposure levels in animals and people. Tissue dose of the toxic moiety is not always directly related to exposure level, especially at the higher exposure levels frequently used in toxicological studies. In these cases, tissue disposition of chemical changes from one exposure level to the next and the basis of this exposure level-dependent behavior must be ascertained to accurately predict response incidence across exposure levels. Because of the need to clearly distinguish the concepts of exposure level and dose to critical tissues, we emphasize the use of the more comprehensive "exposure-dose-response assessment."

The expression "exposure-dose-response assessment" refers to the determination of the quantitative relationship between exposure levels and target tissue dose, and further the relationship between tissue dose and observed/expected responses in animals and people. This portion of the risk assessment process still requires the extrapolation of tissue dosimetry and tissue response observed at high exposure levels to those expected at low exposure levels in test animals, and the extrapolation of toxicologically-equivalent exposure levels from test animals to humans.

In conventional risk analysis for carcinogens, the high exposure level to low exposure level extrapolation is performed using the linearized multistage model (LMS), and interspecies extrapolation is conducted using a conversion factor based on body weight (BW), usually $BW^{2/3}$ or BW^{1}. For systemic toxicants, a reference dose (RfD) or a reference concentration (RfC), an exposure level for humans below which a significant risk of adverse effects is not expected, is derived by dividing the no-observed adverse effect level (NOAEL) in rodents by a safety factor ranging from 10 to 1000. In general, these default extrapolation approaches are used because knowledge of the mechanisms of disposition of the toxicant and the mechanisms by which responses are produced is insufficient to allow more customized approaches. This article examines the progress at CIIT in developing approaches for mechanistic modeling of chemical disposition, of toxicant-target tissue interaction and of tissue response to improve exposure-dose-response assessments for a variety of important chemicals.

Mechanisms in Toxicology

Toxicological research may focus on biological responses observed at various levels of organization, *i.e.*, molecular, cellular, target organ, organism, or population level. At each level of biological organization we speak of the "mechanisms" responsible for these effects. "Mechanism" refers to the critical biological factors that regulate the occurrence of a particular process and the nature of the interrelationships among these factors. Obviously, the mechanisms of interactions at the molecular level are very different from the mechanisms at work on populations. However, in each case mechanism refers to the biological determinants that control the responses at the level of organization. For our purposes, we are interested in the mechanisms by which three particular processes take place: (1) the disposition of chemicals throughout the body, (2) the initial biochemical interaction between chemical and target tissue, and (3) the progressive molecular and

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cellular alterations emanating from the initial interaction leading to toxicity.

Biological Modeling

Biological modeling is the process of developing mathematical descriptions of the interrelationships among the mechanistic determinants of the events of interest. This approach involves (1) identification of the mechanistic determinants (*i.e.*, physicochemical, physiological, biochemical and molecular factors) of the exposure–tissue dose–tissue response continuum (Fig. 1), and (2) encoding the interrelationships among the mechanistic determinants in mathematical equations. The solution of these equations by analytical or numeric methods predicts the behavior of the biological system under a specified set of experimental conditions. The prediction may be related to dosimetry, molecular interactions, or tissue response. The extent to which predictions coincide with subsequent experiments is a test of the "correctness" of the proposed mechanism(s). In this sense, these mathematical models are actually quantitative statements of our proposed hypothesis of toxic action. These models then become central factors in maintaining the risk assessment focus of ongoing research. Experiments, designed based on these working hypotheses, confirm, refute, or alter these hypotheses. In the following sections, we now separate these modeling areas into disposition, interaction with critical target sites, and tissue response, primarily as related to chemical carcinogenesis.

Mechanistic Biological Modeling of Chemical Disposition

Disposition includes absorption, distribution, metabolism and elimination of chemicals. The mechanistic determinants of the disposition of a chemical and its metabolite(s) in an organism include physicochemical (*e.g.*, tissue and blood solubility), biochemical (*e.g.*, kinetic constants for metabolism and protein binding) and physiological factors (*e.g.*, anatomical characteristics, air flow rate, blood flow rate, glomerular filtration rate, tissue volumes, alveolar ventilation rate). The interconnections among these determinants of chemical disposition can be described with a series of differential equations, producing mechanistically-based dosimetry models. Dosimetry modeling efforts at CIIT focus both on locally-reactive and systemic toxicants. The regional dosimetry of inhaled chemicals, such as formaldehyde, is studied with mathematical models of air flow in the upper respiratory tract (Kimbell and Morgan, 1990). Target tissue dosimetry of systemic toxicants

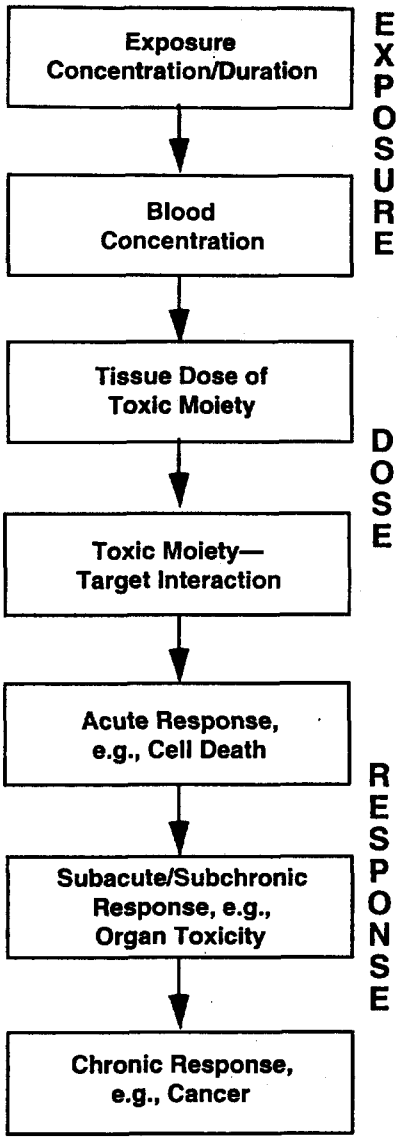

Fig. 1.
The "Exposure-Dose-Response" paradigm for chemical carcinogens and systemic toxicants.

has been described with physiologically-based pharmacokinetic (PBPK) models.

PBPK models consist of a series of anatomically-relevant tissue compartments, each of which receives the chemical via arterial blood and loses the chemical via venous effluent (Fig. 2). The compartments may represent a single tissue, or a grouping of tissues that have similar blood flow and solubility characteristics. In the PBPK models, the rate of tissue uptake of a chemical is typically described either as limited by blood flow or limited by diffusion from blood into the tissue. In the case of blood-flow limited uptake, the rate of change in the amount of a chemical in the tissue (dA_t/dt) is described with a mass balance differential equation, which accounts for the roles of tissue blood flow rates (Q_t), arterio-venous concentration

difference (C_a-C_{vt}), tissue metabolism (dA_{met}/dt) and in certain cases, the production of chemical within the tissue (dPR/dt) (Andersen, 1991):

$$dA_t/dt = Q_t (C_a - C_{vt}) - dA_{met}/dt + dPR/dt$$

The total amount of the chemical in the tissue (A_t) then is given by the integral of this mass balance differential equation. The tissue concentration of the chemical at any time (C_t), can be calculated by dividing the amount in the tissue by tissue volume.

At CIIT, PBPK models are being developed to describe the disposition of several chemicals, including acrylonitrile (Gargas *et al.*, 1990), 2-methoxyethanol (Clarke *et al.*, 1991a), 2,2,4-trimethyl 2-pentanol (Borghoff *et al.*, 1991), methanol (Medinsky *et al.*, 1991), ethylene oxide (Krishnan *et al.*, 1992), 1,3-butadiene (Csanády *et al.*, 1992), and furan (Kedderis *et al.*, 1992). In some cases, the dosimetry modeling focuses on parent chemicals and in other cases, on toxic metabolites. In the case of benzene, current research focuses on incorporating metabolite kinetics and the interaction between the metabolites and/or the parent chemical in determining the overall disposition of benzene and its metabolites (Medinsky and Gargas, 1990). For dioxin and related compounds, the changes in physiological and biochemical parameters due to the growth of the animal and to induction of dioxin-binding proteins resulting from repeated exposures to the chemical have been described within a PBPK modeling framework (Mills *et al.*, 1992). For chloroform, current studies are addressing the importance of route of exposure in determining the disposition kinetics, extent of absorption, and rate of metabolic clearance under various exposure scenarios (Burnett *et al.*, 1992).

The mechanistic, biological basis of the PBPK models permits high exposure to low exposure, interspecies, and route-to-route extrapolation of chemical disposition (Clewell and Andersen, 1985; Krishnan and Andersen, 1991). The principal application of the PBPK models in our research program is in calculating target tissue dose of the chemical or its metabolite.

The next level in the exposure-dose-response paradigm relates to how toxicant-target tissue interactions occur at the molecular level.

Mechanistic Biological Modeling of Toxicant-Target Tissue Interactions

The mechanism of toxicant-target tissue interaction refers to the manner in which the chemical or its metabolite interacts with biological targets, initiating steps critical for ultimate toxicity. This initial interaction usually only constitutes the first of a series of events which together progress in severity to yield overt, irreversible toxic sequelae.

In the chemical carcinogenesis theme at CIIT, three general classes of chemical carcinogens are under investigation: DNA-reactive chemicals, cytotoxic chemicals, and mitogenic chemicals. Mechanistic models of the initial toxicant-target interaction are being developed for prototype chemicals within each class.

For DNA-reactive chemicals, initial target tissue interaction is the formation of DNA adducts. With ethylene oxide, for example, the rate of change in the amount of DNA adducts in the various tissues ($dA_{EO\text{-}DNA}$/dt) of rats and mice is believed to be related to the concentration of the chemical in the tissue ($C_{t\text{-}EO}$), the concentration of DNA in the tissue (C_{DNA}), the volume of the tissue (V_t), the concentration of the adduct at any given time ($C_{EO\text{-}DNA}$), and the rate constants for the formation (k_f) and removal (k_r) of the particular adduct in each tissue:

$$dA_{EO\text{-}DNA}/dt = k_f C_{t\text{-}EO} C_{DNA} V_t - k_r C_{EO\text{-}DNA} V_t$$

The concentration of tissue DNA adducts is determined by dividing the amount of the adduct by tissue volume.

For cytotoxic chemicals, the initial cellular interactions are frequently associated with the delivery/accumulation of sufficient doses of reactive chemical in the tissue to kill individual cells. Some reactive chemicals form long-lived, covalently bound tissue adducts (*e.g.*, carbon tetrachloride, acetaminophen). Other chemicals, such as chloroform, seem to be metabolized to toxic intermediates that are short-lived in the cell. Chloroform is biotransformed in the liver to phosgene and HCl. With chloroform, the high rate of formation of the reactive intermediate is associated with the death of some liver cells. Modeling this process requires mechanistic assertions about metabolism and linking the metabolic rate to cell death by empirical relationships that are not yet fully developed on a mechanistic level. Along these lines, Reitz *et al.* (1990) developed a quantitative description of chloroform-induced cell death based on information on the rate of formation of the reactive metabolite (dA_{met}/dt), number of viable hepatocytes (N_h), volume of the liver (V_l), and the rate at which the cells at risk die (k_{death}):

$$dN_h/dt = - k_{death} SENS\ N_h$$

where SENS = normal or lognormal distribution of cell sensitivity to cytotoxicity as a function of $(dA_{met}/dt)/V_l$. This distribution provides an estimate of the proportion of cells at risk at any time and was empirically derived from *in vivo* experiments.

Dioxin, a tumor promoter in laboratory animals, acts via specific receptors to modulate gene expression, cell growth and differentiation. Among the proteins induced by dioxin is a particular hepatic protein, cytochrome P450IA2, to which dioxin binds. Induction of this binding protein results in a concentration-dependent shift in dioxin to the liver at high exposure levels. The description of dioxin dosimetry includes information about the regulation of gene expression for P450IA2 by dioxin. The interrelationship among the concentration of dioxin in the venous blood leaving the tissue (C_{vt}), the basal ($P450IA2_0$) and maximally induced level

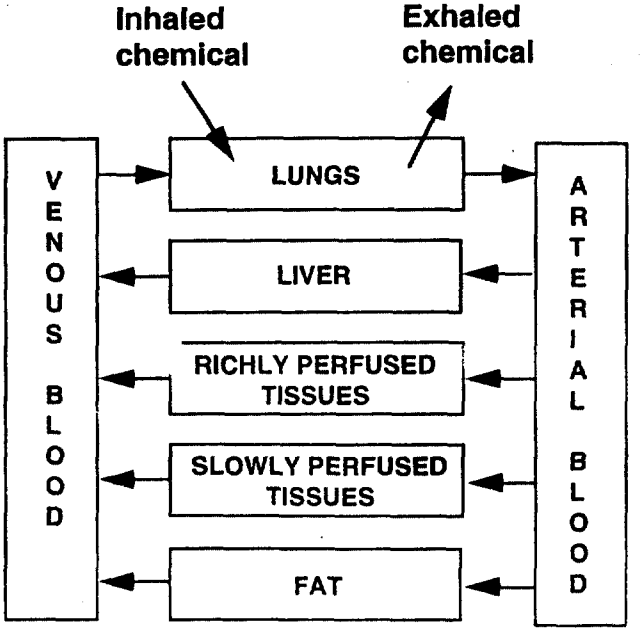

Fig. 2
Structure of a typical physiologically-based pharmacokinetic model for volatile organic chemicals.

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of the gene product ($P450IA2_{max}$), and the *Ah* receptor-dioxin dissociation constant (K_d), has been used to describe the exposure-level-dependence of dioxin accumulation in the liver (Andersen and Greenlee, 1991). The amount of P450IA2 at any time, t, was calculated from the following expression:

$$P450IA2(t) = P450IA2_o + P450IA2_{max}\, C_{vt}/(K_d+C_{vt})$$

P450IA2 is not expected to be directly associated with toxic or promotional efficacy of dioxin. Modulation of growth regulatory genes, though, can also be quantitatively examined by similar strategies and levels of these gene products linked to the effects of dioxin on cell growth and differentiation. More recent descriptions of dioxin induction of P450IA2 have included ternary complexes between the *Ah* receptor, dioxin and DNA-binding sites (Mills *et al.*, 1992).

In each example above, the toxicant-target interaction is described mathematically by equations that include the critical determinants of dosimetry and initial tissue interactions. For interspecies extrapolation of the mechanism of action of a chemical, quantitative information on the critical biological determinants (*e.g.*, the rates of formation and degradation of macromolecular adducts and the relative sensitivity of cells from different species) needs to be obtained for the animal species of interest. Quantitative information about these critical determinants can frequently be obtained by using cells or tissues *in vitro* to measure adduct formation rates, sensitivity of hepatocytes to cell killing, or dose-response of enzyme induction. Once these *in vitro* techniques are determined to faithfully predict *in vivo* interactions in animals, similar *in vitro* studies using human tissues can be performed to provide the required parameters for the models to conduct interspecies extrapolations.

Thus, biologically-based models of toxicant-target tissue interaction provide both a clear focus for experiments on the mechanisms of action with emphasis on extrapolation and link the tissue dosimetry of the toxic moiety to early biological sequelae of the toxicant interaction.

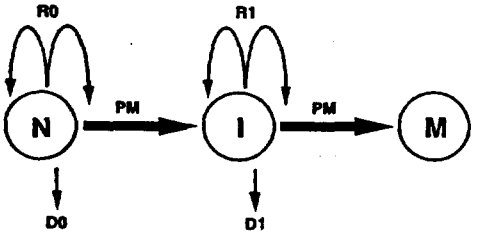

Fig. 3
Biologically-based cancer response model. In this model, a normal cell (N) goes through two, step-wise mutational events (PM) to become a malignant cell (M). R and D represent the replication and death rates of the normal (0) and intermediate (1) cells.

ROLE OF TOXICANT-TARGET TISSUE INTERACTIONS IN CHEMICAL CARCINOGENESIS AND IN THE EXPRESSION OF OTHER TOXIC ENDPOINTS

Type of toxicant-tissue interaction	Example	Type of possible carcinogenic effects	Other toxic endpoints caused by same interaction
DNA-reactivity	Ethylene oxide	Initiator	Reproductive and developmental toxicity
Cytotoxicity	Chloroform	Initiator(?)/ Promoter	Hepatic and renal toxicity
Stimulation of cell growth	TCDD	Promoter	Teratogenic/other organ-level toxicity

Mechanistic Biological Modeling of Tissue Response

The interaction of toxic chemicals with biological macromolecules may initiate cellular changes that lead to measurable toxic responses. DNA adducts may cause mutations; cytotoxic metabolites can kill individual cells, leading to enhanced cell proliferation for tissue repair; and expression of growth factors can act as a direct proliferation stimulus. The quantitative influence of each of these processes on tumor outcome can be described with biologically-based tissue response models.

Two-stage biologically-based cancer models, such as the Moolgavkar-Venzon-Knudson (MVK) model (Fig. 3), represent carcinogenesis as an end result of two critical events that correspond to irreversible genetic changes (mutations)— one converting a normal cell to an intermediate cell genotype and a second converting the intermediate cell to an overtly malignant genotype (Moolgavkar and Venzon, 1979; Moolgavkar and Knudson, 1981). This MVK formulation accounts for the growth of normal and intermediate cell populations over time. The model adequately describes the incidence of certain human cancers, such as retinoblastoma, where the cancer is manifest after the loss of function of both alleles of a tumor suppressor gene in the malignant cell (Moolgavkar, 1986).

Much more research is necessary to determine if this model is an adequate description of the mechanisms underlying chemically induced tumors, but the MVK description certainly provides a basis for developing testable hypotheses and provides a vehicle for introduction of biological data into the exposure-dose-response analysis for toxicants (Thorslund *et al.*, 1987; Conolly *et al.*, 1992). The parameters of the MVK model are interpretable in biological terms. For example, the model parameters D and R (Fig. 3) are altered, respectively, by cytotoxicants (*e.g.*, chloroform) and promoters (*e.g.*, dioxins), whereas PM is increased by DNA-reactive chemicals (*e.g.*, ethylene oxide, butadiene epoxide, cyanoethylene oxide).

At CIIT, the biologically-based response modeling approach has been used to simulate the growth of putatively preneoplastic foci of altered growth in rat liver. Simulations have been developed both for spontaneously-occurring foci (Conolly *et al.*, 1991) and for foci in rats initiated with diethylnitrosamine and promoted with Wy-14,643 (Marsman *et al.*, 1991). The modeling indicated that the effects of Wy-14,643 on both cell division and death rates accounted for the observed growth of foci.

Compared to biologically-based modeling of chemical disposition and of initial toxicant-target tissue interaction, biologically-based modeling of tissue responses to toxic agents is in its infancy (Conolly and Andersen, 1991). The lag in response modeling is due in part to the more complicated nature of biological events involved in cancer and other well-developed pathological effects. This may also be a reflection of the persistent notion that tissue response models can only be created once all the data are available and there is widespread agreement on the mechanism(s) of toxicity. In practice, this takes a long time and, in fact, one never has "perfect" knowledge. Therefore, these models need to be developed early on in the research program from available data and used as research tools to identify data gaps and prioritize research.

There is often a tension between model building activities and mechanistic research. Many scientists would argue that these two functions are separate from one another, *i.e.*,

mechanistic research provides from a distance the grist which is required to generate the models. This independence of the two activities represents one approach, but it is quite inefficient. Ideally, the goal should be to use these models (*i.e.*, the quantitative hypothesis of action) to assist in selecting, prioritizing and designing those mechanistic studies that are most likely to provide answers to questions/issues that must be resolved in order to conduct risk assessment for a chemical. In essence, integration of the available data into a model efficiently identifies data gaps. These data gaps may be related to specific experiments designed to obtain quantitative values for metabolic rates, concentration of specific gene products, etc., or be related to areas in which clear mechanistic hypotheses are vague or absent. For instance, what are the cellular events required for cell death from reactive metabolites of chloroform, or the events required to yield a mitotic event following treatment with dioxin? These quantitative models in turn are enriched, altered, or overthrown by the accumulation of new results from the model-directed experiments.

Mechanistically- and Biologically-Based Risk Assessment

The major advantage in combining mechanistic toxicology studies with a modeling framework is to increase the likelihood that results from these studies will eventually impact human health risk assessment. In the past, and even today, most mechanistic toxicology studies tend to be qualitative and fall into the extreme left side of the sphere shown in Fig. 4. Integration of mechanistic research with quantitative biological modeling imparts a more direct risk assessment orientation to the research and focuses attention on the intersection of the two spheres of activity. An integrated program becomes more efficient in use of scarce resources and more focused on human health risk assessment endpoints.

The goal of virtually all research activities at CIIT is to develop mechanistic information

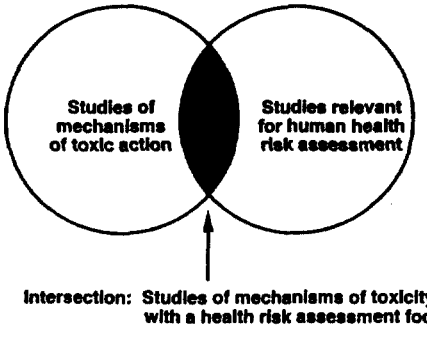

Fig. 4
Approach for selecting mechanistic studies required for conducting quantitative risk assessment for a chemical.

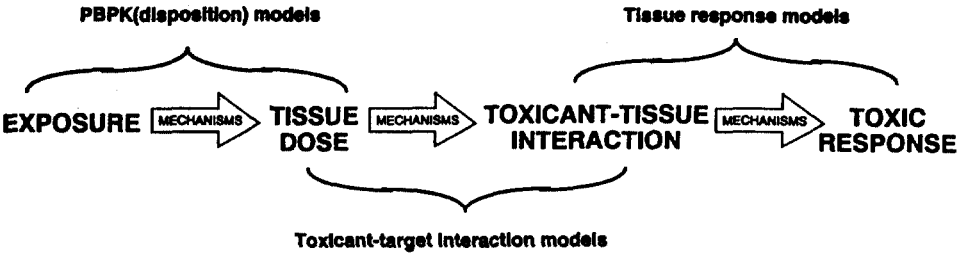

Fig. 5
Schematic representation of the use of biologically-based models in linking the mechanisms of the exposure-dose-response continuum.

to permit improved exposure-dose-response assessments for important prototype chemicals. The operative strategy is to incorporate all mechanistic data within a series of "seamless" exposure-dose-response models such that each research activity becomes linked to the downstream and upstream elements of the overall exposure-dose-response paradigm.

This mechanistic research/biological modeling approach involves the sequential linking of the three types of quantitative models (*i.e.*, disposition, toxicant-target tissue interaction, and tissue response) through knowledge of the appropriate chemical and biological determinants (Fig. 5).

In doing so, the dosimetry model translates the external exposure concentration of a chemical into the transient or cumulative concentration of the toxic moiety in the target tissue. The toxicant-target tissue interaction model uses the dosimetry information in mathematical equations to provide an estimate of the extent of the relevant tissue events that ensue. The sequential linkage of the toxicant-target interaction model with the cancer response model is via cell growth and/or mutation frequency which appear(s) directly in the differential equations describing tumor development. The hypotheses for the exposure-dose-response relationships are now captured in a set of linked equations that calculate tumor incidence for any exposure concentration of a chemical (Fig. 6).

A quantitative cancer risk assessment strategy based on the mechanistic biological modeling approach outlined in this article has recently been formulated for formaldehyde, a chemical for which considerable data base has been generated at the Institute (Conolly *et al.*, 1992). Specifically, in this case, predicted target tissue DNA-protein crosslinks (DPX) resulting from inhaled formaldehyde in humans are to be used in conjunction with the relationship between DPX and cell replication for the rat to estimate expected human cell replication. This comprehensive modeling approach for estimating human cancer risk from formaldehyde exposure will thus incorporate scaled pharmacokinetics and an MVK description configured for the rat.

Tissue response modeling need not be restricted to cancer as an endpoint. In fact, the toxicant-target tissue interactions integral to the research activities in chemical carcinogenesis at CIIT are also central in describing the onset of other toxic sequelae of regulatory importance (Table). Even when the mechanism of tissue response at the molecular level is not clearly understood, the quantitative relationship between tissue dosimetry of the toxic moiety of a chemical and tissue response can be examined. This approach enables the correlation of toxic effects with the internal dose of the toxic moiety estimated with PBPK models, rather than relying on the environmental/exposure concentration of the parent chemical as the dose surrogate in relating the exposure and response to conduct quantitative risk assessment (Andersen *et al.*, 1987). Such an approach is being used at CIIT to assess the teratogenic risks associated with exposure to 2-methoxyethanol (Clarke *et al.*, 1991b).

Mechanistic Biological Modeling in Toxicology Research

Biologically-based modeling offers an opportunity to effectively integrate individual mechanistic research activities (*e.g., in vitro* metabolism, pharmacokinetics, macromolecular interaction, oncogene activation, pathogenesis) within a predictive quantitative framework. A major advantage of these models is that they allow the evaluation of the various plausible hypotheses by computer simulation. We can ask questions of the "if . . . then" nature. For example, if the model structure is correct and the rate of a reaction or another process is varied, then what is the expected impact on the final tissue response? The model can be used to generate quantitative predictions of the expected experimental outcome based on the working hypotheses of the experimentalists, and the most attractive hypothesis based on the simulations is then tested experimentally. If the experimental results differ from the predicted outcome, then the model has to be modified. Just as our ideas change with new experimental data/observations, these biological models are dynamic constructs that can be continually updated by new information and the revised

(Continued on page 6)

models used, in turn, as guides in another iteration for designing new research studies.

This model-based approach for improving exposure-dose-response assessment is really nothing more than a general systems approach to solving complex interdisciplinary problems in toxicology. As noted by Kac (1969): "The main role of modeling is not so much to explain and predict— though ultimately these are the main functions of science— as to polarize thinking and to pose sharp questions."

For a risk assessment orientation in toxicology research, these sharp questions pertain to how the individual elements of the exposure-dose-response relationship inform us of the mechanistic basis for extrapolating observations in animals to humans and how to design new studies to estimate the likely sensitivity of people.

In summary, the biologically-based modeling approach enables the identification, characterization and integration of the mechanistic determinants of chemical disposition, toxicant-target tissue interactions, and tissue responses into a quantitative simulation model of toxic processes. The development of such integrated mechanistic biological models of the exposure-dose-response continuum should enhance our ability to design mechanistic toxicology studies that maintain a human health risk assessment focus, and to integrate these aspects more fully into the risk assessment process.

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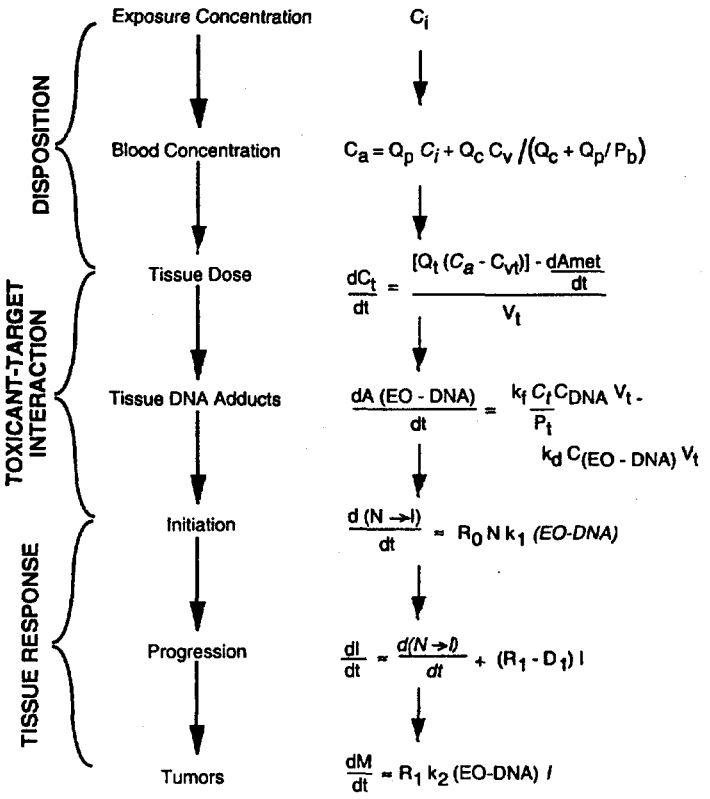

Fig. 6

Quantitative description of the exposure-dose-response continuum to estimate tumor incidence for any exposure concentration of a volatile organic chemical (EO) detoxified by metabolism, based on the working hypotheses of its mechanisms of disposition, target interaction (*e.g.*, DNA reactivity) and tissue response. The terms linking any two steps in the process are italicized. Concentration of the chemical in arterial blood (C_a) is determined from the interrelationship between the breathing rate (Q_p), inhaled concentration (C_i), cardiac output (Q_c), venous blood concentration (C_v) and the blood:air partition coefficient (P_b). Rate of change in the tissue concentration of the chemical (dC_t/dt) is calculated from the tissue blood flow rate (Q_t), arteriovenous concentration difference (C_a-C_{vt}), the rate of metabolism (dA_{met}/dt) and the volume of the tissue (V_t). The rate of change in the amount of the DNA adducts formed by the chemical ($dA_{(EO\text{-}DNA)}/dt$) is represented by the difference between the rate of formation (a function of constant (k_f), concentration of the chemical in the venous blood leaving the tissue (C_{vt}), volume of the tissue (V_t) and the tissue concentration of DNA (C_{DNA})) and degradation (a function of the adduct removal rate constant (k_d), concentration of the adduct at anytime ($C_{(EO\text{-}DNA)}$) and the volume of the tissue (V_t)). The cancer response arising from exposure to this chemical is hypothesized to be the end result of two critical events ($N \rightarrow I$; $I \rightarrow M$) that correspond to mutations, one converting the normal cell (N) to an intermediate cell (I) and the second mutation converting the intermediate cell to a malignant genotype (M). Birth rates of normal and intermediate cells are R_0 and R_1 and death rates are D_0 and D_1, and the mutation frequencies between cell types are represented as a function of the DNA adduct levels (k_x(EO-DNA)). Predicted cancer incidence is a stochastic function of the numbers of these various cell types over time.

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The Authors

Dr. Melvin E. Andersen joined CIIT in 1989 from the Armstrong Aerospace Medical Research Laboratory, WPAFB, Ohio, where he had worked for 10 years. His research interests emphasize the development of quantitative physiological models for tissue dosimetry and tissue response for a variety of environmental and occupational chemicals and the use of these models for human health risk assessment.

Dr. Kannan Krishnan received his Ph.D. in Environmental Toxicology from the University of Montreal. He was Assistant Professor of Ecotoxicology at Concordia University, Montreal, before joining CIIT as a Postdoctoral Fellow in May 1990. His research interests include the development of approaches for mechanistic modeling of multiple chemical interactions.

Dr. Rory B. Conolly joined CIIT in the fall of 1989. His work at the Institute centers on the development of computer simulation models of the molecular, cellular, and tissue processes involved in cytotoxicity and carcinogenicity.

Dr. Roger O. McClellan, President and CEO of CIIT since 1988, has had a long-standing interest in integrating data from *in vitro*, animal, and epidemiological studies within an exposure→dose→response paradigm to obtain estimates of human health risks of exposure to radiation and chemicals. He has a special interest in estimating the health risks of exposure to airborne chemicals.

The authors, left to right: Krishnan, Andersen, Conolly, and McClellan

Presentations and Interactions

Bond, J. A. Participant, Meeting of the National Institutes of Health Toxicology Study Section and NIH Staff, including the Director of NIH; Bethesda, MD, January 7.

Conolly, R. B."Cancer and Non-Cancer Risk Assessments— Not So Different If We Consider Mechanisms: Formaldehyde and Chloroform," Meeting of the Research Triangle Chapter of the Society for Risk Analysis, Research Triangle Park, NC, January 23.

Gerard, J. Participant, Meeting of the North Carolina Supercomputing Center Advisory Council, Research Triangle Park, NC, January 10.

Goldsworthy, T. L. "Research Program at CIIT," Allied-Signal, Inc., Occupational Health Meeting, Copper Mountain, CO, January 21.

Kimbell, J. S. Participated as Member, Meeting of Regional Coordinators of the Women and Mathematics Program, Joint Mathematics Meetings, Baltimore, MD, January 9-10.

Krishnan, K. "Pollution: Causes and Solutions," Scientist-Teacher Partnership Program, Bethesda Elementary School, Durham, NC, January 31.

McClellan, R. O. Participated in Meeting of Executive Committee, Science Advisory Board, U. S. Environmental Protection Agency, Washington, DC, January 8.

McClellan, R. O. Participated as Member, Meeting of the Research Committee, Health Effects Institute, Cambridge, MA, January 22-23.

McClellan, R. O. Participated as Vice President, Meeting of the Board of Directors of the American Association for Aerosol Research, Pasadena, CA, January 25..

Preston, R. J. Appointed to Scientific Committee 1-4 on Extrapolation of Risks frm Nonhuman Experimental Systems to Man, National Council on Radiation Protection and Measurements, January 1992.

Cell Proliferation Conference at NIEHS

CIIT was one of four co-sponsors of an international meeting on cell proliferation and chemical carcinogenesis, held at the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, NC, January 14-16. Other sponsors were NIEHS, the International Life Sciences Institute–Risk Science Institute, and the American Industrial Health Council.

CIIT Vice President Dr. James A. Popp served on the Scientific Program Committee and also acted as Chairperson of Session III, Cell Proliferation and Modeling of Organ-Specific Carcinogenesis, on January 15. Mrs. Edna Mangum of CIIT was a conference coordinator. Two CIIT staff scientists made platform presentations: Dr. Thomas L. Goldsworthy spoke on "Labeling Procedures and Design of Cell Proliferation Studies Relating to Carcinogenesis" on January 14, and Dr. Kevin T. Morgan discussed "Cell Proliferation and Nasal Cavity Carcinogenesis" on January 15.

The Institute's research in the area of chemically-induced cell proliferation represented approximately 30% of the 37 posters displayed. The following CIIT poster abstracts appeared in the Program:

Butterworth, B. E., Sprankle, C. S., Goldsworthy, S. M., Popp, J. A., Wilson, D. M., and Goldsworthy, T. L. "Evaluation of Genotoxicity, Cytotoxicity, Cell Proliferation and Gene Expression in Livers of Rats and Mice Treated with Furan."

Eldridge, S. R., Butterworth, B. E., Dunn, C. S., Popp, J. A., and Goldsworthy, T. L. "Use of Bromodeoxyuridine in the Measurement of Chemically-Induced Cell Proliferation."

Eldridge, S. R., Goldsworthy, T. L., Popp, J. A., and Butterworth, B. E. "Mitogenic Stimulation of Hepatocellular Proliferation in Rodents During 90-Day 1,4-Dichlorobenzene Administration."

Eldridge, S. R., Lyght, O., Butterworth, B. E., and Goldsworthy, T. L. "Comparison of Proliferating Cell Nuclear Antigen, Bromodeoxyuridine and ^{3}H-Thymidine for Measurements of Chemically-Induced Hepatocellular Proliferation in Mice and Rats."

Foley, J., Ton, T., Maronpot, R., Butterworth, B. E., and Goldsworthy, T. "Comparison of Proliferating Cell Nuclear Antigen (PCNA) and Tritiated Thymidine([^{3}H]-TdR) as Markers of Proliferating Hepatocytes in Rats."

Goldsworthy, S. M., Goldsworthy, T. L., Sprankle, C. S., and Butterworth, B. E. "Protooncogene Expression Associated with Induced Cell Proliferation in the F344 Rat Liver."

Gross, E. A., Mellick, P., Kari, F., and Morgan, K. T. "Histopathology and Cell Replication in the Nasal Epithelium of Rats and Mice Exposed to Glutaraldehyde Vapor."

Larson, J. L., Wolf, D. C., Gargas, M. L., and Butterworth, B. E. "Acute Hepato- and Nephrotoxicity of Chloroform ($CHCl_3$) in Female B6C3F1 Mice and Male Fischer-344 Rats."

Marsman, D., Goldsworthy, T., and Popp, J. "Selective Promotion of Altered Hepatic Foci and Hepatocellular Carcinomas by Wy-14,643 and Clofibric Acid in Comparison to Phenobarbital."

Monticello, T. M., Miller, F. J., Swenberg, J. A., Starr, T. B., Gibson, J. E., and Morgan, K. T. (1992). "Association of Enhanced Cell Proliferation and Nasal Cancer in Rats Exposed to Formaldehyde."

Monticello, T. M., and Morgan, K. T. "Formaldehyde-Induced Pathology and Cell Proliferation in the Respiratory Tract: Comparison of F344 Rats with Rhesus Monkeys."

Kimbell, Two Fellows Join Staff in January

Julia S. Kimbell joined the Institute January 1 as Applied Mathematician. Dr. Kimbell, who received a Ph.D. in differential geometry from Duke University in 1988, recently completed a postdoctoral fellowship at CIIT. She holds B.A. and M.A. degrees from Middlebury College and Duke University, respectively.

Dr. Kimbell continues her research on the pathogenesis of toxicant-induced upper respiratory tract disease. Her primary focus is on upper respiratory tract airflow and transport of reactive gases in relationship to site-specific lesion development. In addition to performing experimental studies, she is developing computer simulation algorithms for execution on the supercomputer and and the Institute's workstations. Dr. Kimbell is also collaborating with other staff members on stochastic modeling of carcinogenic processes.

Two Postdoctoral Fellows began appointments in January. Susan C. Sisk received a Ph.D. in biochemistry from the University of Virginia, where her graduate studies centered on xenobiotic induction of glutathione transferase genes in mouse tissues and cell lines. Dr. Sisk will be working with Dr. Les Recio to analyze formaldehyde-induced alterations in the p53 tumor suppressor gene. In addition, she will determine the *in vivo* mutagenicity of ethylene oxide using a transgenic mouse mutation assay.

Andrew Standeven joins the laboratory of Dr. Tom Goldsworthy, where his research will focus on hepatocarcinogenicity of unleaded gasoline in mice. A National Science Foundation Predoctoral Fellow, Dr. Standeven received a Ph.D. in pharmacology and toxicology from Dartmouth University this year. His dissertation research examined the role of glutathione and ascorbate in chromium (II) metabolism and toxicity in rats.